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博 士 学 位 论 文

构建阿霉素核-H 亚基铁蛋白且用于药效分析、抑制 HepG2 细胞生长
的差异蛋白质组学研究

Construction of Adriamycin Core-Human H Subunit Ferritin
for Analysis of Its Drug Effective and Differential
Proteomics of HepG2 Cell Line During Inhibition Growth

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目 录

摘 要	IX
Abstract	XI
缩略语	XIII
1 前 言	1
1.1 铁蛋白	1
1.1.1 铁蛋白的发现与组成	1
1.1.2 铁蛋白的表达调控	2
1.1.3 血清铁蛋白	3
1.1.4 铁蛋白膜受体	3
1.2 纳米药物载体与纳米铁蛋白靶向给药系统	4
1.3 转铁蛋白受体 1 (TfR1)	6
1.4 阿霉素	7
1.5 顺铂	11
1.5.1 CDDP 在肿瘤治疗中的作用及抗癌机制	11
1.5.2 肿瘤细胞对 CDDP 产生耐药性的机制	11
1.6 蛋白质组学	12
1.6.1 蛋白质组学的含义及其研究内容	12
1.6.2 蛋白质组学研究的核心技术	13
1.6.3 双向凝胶电泳	13
1.6.4 质谱技术	14
1.6.5 鸟枪法蛋白质组学	14
1.6.6 定量蛋白质组学	15
1.6.7 生物质谱技术	15
1.6.8 质量分析器的分类与串联应用	17
1.7 本文的研究内容与意义	19
2 铁蛋白 H 亚基的原核表达与 H 亚基铁蛋白的构建	20
2.1 材料与方法	20

2.1.1	材料、试剂与耗材	20
2.1.2	实验仪器设备	22
2.1.3	方法	22
2.2	结果与讨论	46
2.2.1	人铁蛋白 H 亚基原核表达载体的构建	46
2.2.2	三种表达载体在不同浓度 IPTG 诱导条件下的表达情况	47
2.2.3	pGEX-4T-1-FTH1 表达菌种的筛选和表达条件的优化	47
2.2.4	GST-人铁蛋白 H 亚基融合蛋白的表达、纯化与 GST 标签的切除	47
2.2.5	原核表达的人铁蛋白 H 亚基肽质量指纹鉴定	47
2.2.6	缺铁核人 H 亚基铁蛋白的透射电镜成像	50
2.3	小结	52
3	阿霉素核-H 亚基铁蛋白 (D-HFt) 的构建及其体内外成像	53
3.1	材料与方法	53
3.1.1	材料、试剂与耗材	53
3.1.2	实验仪器设备	53
3.1.3	方法	54
3.2	结果与讨论	60
3.2.1	D-HFt 的阿霉素包装量	60
3.2.2	D-HFt 对于 HepG2 细胞 24 h 的生长抑制情况	61
3.2.3	FITC 标记的 HFt 结合 HepG2 细胞的荧光显微镜观察结果	61
3.2.4	荷瘤裸鼠活体与离体成像结果	62
3.2.5	裸鼠异体移植人肝癌的治疗结果	63
3.3	小结	69
4	阿霉素核-H 亚基铁蛋白与阿霉素抑制 HepG2 细胞生长的差异蛋白质组学研究	71
4.1	材料与方法	71
4.1.1	材料、试剂与耗材	71
4.1.2	实验仪器设备	71
4.1.3	方法	72

4.2	结果	79
4.2.1	肽质量指纹图谱搜索与蛋白鉴定结果	79
4.2.2	鉴定蛋白的 GO 注释结果	80
4.2.3	鉴定蛋白的非标记定量	89
4.2.4	部分差异表达蛋白 mRNA 转录水平的 Real-time PCR 检测	93
4.3	讨论	93
4.3.1	CFL1 蛋白	94
4.3.2	STMN1 蛋白	95
4.3.3	TBX4 蛋白	97
4.3.4	CDC25 磷酸酶与 CDC25B 磷酸酶	97
4.3.5	ELFN2 蛋白	99
4.3.6	PDHX 蛋白	99
4.3.7	PRDM16 蛋白	100
4.3.8	REST 蛋白	100
4.3.9	AKAP9 蛋白	103
4.4	小结	105
5	纳米顺铂核-H 亚基铁蛋白与顺铂抑制 HepG2 细胞生长的差异蛋白质组学研究	106
5.1	材料与方法	106
5.1.1	材料、试剂与耗材	106
5.1.2	实验仪器设备	106
5.1.3	方法	107
5.2	结果	108
5.2.1	纳米顺铂核人 H 亚基铁蛋白 (NCC-HFt) 的顺铂包装量	108
5.2.2	肽质量指纹图谱搜索与蛋白鉴定结果	109
5.2.3	鉴定蛋白的 GO 注释结果	110
5.2.4	鉴定蛋白的非标记定量	124
5.2.5	部分差异表达蛋白 mRNA 转录水平的 Real-time PCR 检测	124
5.3	讨论	125

5.3.1	AHSG 蛋白	125
5.3.2	ALDOA 蛋白	127
5.3.3	FUBP1 蛋白	128
5.3.4	不均一性核糖核蛋白家族	130
5.3.5	LASP1 蛋白	133
5.3.6	细胞角蛋白家族	135
5.3.7	NonO 蛋白	136
5.4	小结	137
展 望	138
参考文献	139
在学期间发表的论文	166
致 谢	167

Content

Chinese Abstract	IX
English Abstract	XI
Abbreviations	XIII
1 Introduction	1
1.1 Ferritin	1
1.1.1 The discovery history and composition of ferritin	1
1.1.2 Regulation of ferritin expression	2
1.1.3 Serum ferritin	3
1.1.4 Ferritin membrane receptor	3
1.2 Nano drug carrier and nano ferritin drug targeting delivery system	4
1.3 Transferrin receptor 1 (TfR1)	6
1.4 Doxorubicin	7
1.5 Cisplatin	11
1.5.1 The function and anti-cancer mechanism of cisplatin in tumor treatment	11
1.5.2 The mechanism of tumor cells to cisplatin resistance	11
1.6 Proteomics	12
1.6.1 The definition and research contents of proteomics	12
1.6.2 Key techniques of proteomics	13
1.6.3 Two-dimensional electrophoresis	13
1.6.4 Mass-spectrometric techniques	14
1.6.5 Shotgun proteomics	14
1.6.6 Quantitative proteomics	15
1.6.7 Biomass spectrometry	15
1.6.8 The classification of mass analyzers and tandem mass spectrometry applications	17
1.7 Significance and purpose of this study	19
2 The expression of human ferritin H subunit in <i>E. coli</i> and	

	construction of combinant human H-ferritin	20
2.1	Materials and Methods	20
2.1.1	Materials, reagents and consumables	20
2.1.2	Instruments	22
2.1.3	Methods	28
2.2	Results and discussion	46
2.2.1	The construction of human ferritin H subunit recombinant expression vectors	46
2.2.2	The expression results of three vectors using diffeerent concentraion IPTG induction	47
2.2.3	Screening pGEX-4T-1-FTH1 recombinants and expression conditions optimization	47
2.2.4	Expression, purification and removal of GST tag from GST-human ferritin H subunit fussion protein	47
2.2.5	PMF identification of recombinant human ferritin H subunit	47
2.2.6	TEM imaging of recombinant human H-apoferritin	50
2.3	Summary	52
3	Construction, <i>in viov</i> and <i>ex vivo</i> imaging of doxorubicin core recombinant human H-ferritin (D-HFt)	53
3.1	Materials and Methods	53
3.1.1	Materials, reagents and consumables	53
3.1.2	Instruments	53
3.1.3	Methods	54
3.2	Results and discussion	60
3.2.1	Doxorubicin loading rate of D-HFt	60
3.2.2	Growth inhibition results of HepG2 cells after D-HFt treatment 24 h	61
3.2.3	Fluorescence microscope observation results of FITC labled D-HFt binding and entering to HepG2 cells	61
3.2.4	<i>In viov</i> and <i>ex vivo</i> imaging results of nude mice	62

3.2.5	Therapy results of HepG2 subcutaneous tumor models in nude mice	63
3.3	Summary	69
4	Differential expression proteomics study of HepG2 cells growth inhibition treated by D-HFt and doxorubicin	71
4.1	Materials and Methods	71
4.1.1	Materials, reagents and consumables	71
4.1.2	Instruments	71
4.1.3	Methods	72
4.2	Results	79
4.2.1	PMF search and protein identification results	79
4.2.2	Gene ontology annotation results of identified proteins	80
4.2.3	Lable-free quantification results of identified proteins	89
4.2.4	Real-time PCR assay results of partial differential expression proteins' transcript levels	93
4.3	Discussion	93
4.3.1	Protein CFL1	94
4.3.2	Protein STMN1	95
4.3.3	Protein TBX4	97
4.3.4	CDC25 phosphatase and CDC25B phosphatase	97
4.3.5	Protein ELFN2	99
4.3.6	Protein PDHX	99
4.3.7	Protein PRDM16	100
4.3.8	Protein REST	100
4.3.9	Protein AKAP9	103
4.4	Summary	105
5	Differential expression proteomics study of HepG2 cells growth inhibition treated by NCC-HFt and CDDP	106
5.1	Materials and Methods	106
5.1.1	Materials, reagents and consumables	106

5.1.2	Instruments	106
5.1.3	Methods	107
5.2	Results	108
5.2.1	Cisplatin loading rate of NCC-HFt	108
5.2.2	PMF search and protein identification results	109
5.2.3	Gene ontology annotation results of identified proteins	110
5.2.4	Lable-free quantification results of identified proteins	124
5.2.5	Real-time PCR assay results of partial differential expression proteins' transcript levels	124
5.3	Discussion	125
5.3.1	Proteins AHSG	125
5.3.2	Proteins ALDOA	127
5.3.3	Proteins FUBP1	128
5.3.4	Heterogeneous nuclear ribonucleoprotein family	130
5.3.5	Proteins LASP1	133
5.3.6	Cytokeratin family	135
5.3.7	Proteins NonO	136
5.4	Summary	137
	Prospection	138
	References	139
	Publications	166
	Acknowledgements	167

摘 要

人铁蛋白由于具有独特的纳米笼状结构、合适的大小、良好的生物安全性以及易于通过化学或遗传学的方法进行表面改造等特性而成为理想的肿瘤靶向给药载体,因而受到了科学家们的广泛关注。本文构建了 pET-21b-FTH1、pET-28a-FTH1 和 pGEX-4T-1-FTH1 三种人铁蛋白 H 亚基原核表达载体,经检测,三种表达载体中 pGEX-4T-1-FTH1 的表达效率最高,且在 0.3 mM IPTG、25 °C 条件下诱导表达能够同时兼顾表达效率和融合蛋白溶解性。经 SDS-PAGE 与 PMF 鉴定, pGEX-4T-1-FTH1 表达的重组融合蛋白在切除 GST 标签后确为人铁蛋白 H 亚基。而且,经透射电子显微镜观察,重组人铁蛋白 H 亚基能够在生理条件下自发组装成缺铁核人 H 亚基铁蛋白 (HFt) 的纳米笼状结构。

阿霉素与顺铂均为临床上治疗肿瘤的一线用药,但它们都存在半衰期短、副作用大等不足。本文首先构建了阿霉素核-H 亚基铁蛋白 (D-HFt),其阿霉素包装量约为 24.8% (质量分数)。荧光显微镜观察显示, FITC 标记的 HFt 能与 HepG2 细胞靶向结合并进入细胞的内部。异体移植 HepG2 肿瘤的裸鼠活体 (*in vivo*) 与离体 (*ex vivo*) 成像显示,近红外荧光染料 Cy7.5 标记的 D-HFt 在荷瘤裸鼠体内的半衰期较阿霉素要长得多,且 D-HFt 具有很好的肿瘤靶向性。异体移植 HepG2 肿瘤裸鼠的治疗实验显示,在同等给药剂量和给药频率的条件下, D-HFt 对肿瘤生长的抑制情况优于阿霉素。同时,治疗后荷瘤裸鼠主要器官石蜡切片的苏木精-伊红染色显示未观察到 D-HFt 对荷瘤裸鼠的主要器官造成明显的病理异常如出血、水肿、淋巴细胞浸润、坏死等现象。

液相色谱-质谱联用 (LC-MS) 技术是目前蛋白质组学研究的主要技术手段之一。本文利用 LC-MS 对 D-HFt 与阿霉素作用于 HepG2 细胞 24 h 后的差异表达蛋白质组进行了研究,找到了 37 个差异表达的蛋白。其中 EEF1A1 等 19 个蛋白在 D-HFt 组中表达上调, CFL1 等 18 个蛋白表达下调。这些结果为进一步研究 D-HFt 较阿霉素具有更好的疗效所蕴含的分子机制奠定了基础。

另外,本文还构建了纳米顺铂核-H 亚基铁蛋白 (NCC-HFt),并利用 LC-MS 对 NCC-HFt 与顺铂作用于 HepG2 细胞 24 h 后的差异表达蛋白质组进行了研究,

找到了 24 个差异表达的蛋白。其中 AHSB 等 19 个蛋白在 NCC-HFt 组中表达上调，ALDOA 等 5 个蛋白表达下调。这些结果有利于进一步研究 NCC-HFt 所具有的缓释作用对疗效的影响。

关键词：铁蛋白；靶向给药；蛋白质组学

Abstract

Human ferritin has unique nanocage structure, appropriate size, excellent biosafety and easily modified surface with either genetically or chemically methods. Because of these characters, it became an ideal tumor targeting drug delivery carrier, and focused by many scientists. In this thesis, we constructed three expression vectors, pET-21b-FTH1, pET-28a-FTH1 and pGEX-4T-1-FTH1, for expressed human ferritin H subunit in *E. coli*. After tested these three vectors, we found pGEX-4T-1-FTH1 was the most efficient expression vector in them, and 0.3 mM IPTG with 25 °C were the optimized induction conditions for its expression. These conditions could balance expression efficiency with solubility of the recombinant fusion protein. After identified with SDS-PAGE and PMF, we were sure it was human ferritin H subunit when GST tag removed from the recombinant fusion protein expressed by pGEX-4T-1-FTH1 using thrombin cleavage. Furthermore, we observed in TEM images that recombinant human ferritin H subunits had self-assembled to human H-ferritin (HFt) nanocages under physiological conditions.

Doxorubicin and cisplatin are both first-line chemotherapy drug for tumor treatment. But they all have short circulation half-life and strong side effects. In this thesis, we constructed doxorubicin core recombinant human H-ferritin (D-HFt), which doxorubicin loading rate was about 24.8% (wt%). Fluorescence microscopy observation demonstrated FITC labeled HFt can bind to HepG2 cells and internalized by cells. When studied on HepG2 subcutaneous tumor models in nude mice, these D-HFt showed a longed circulation half-life, higher tumor uptake, better tumor growth inhibition, and less side effects than free doxorubicin. Besides, H&E staining found necrosis in the tumors treated with D-HFt, but no obvious pathological abnormalities such as hemorrhage, edema, lymphocyte infiltration and necrosis in major organs including the heart.

Liquid chromatography-mass spectrometry (LC-MS) technique is one of the main

methods using in proteomics research at present. We studied differential expressed proteome after treated HepG2 cells with D-HFt or doxorubicin 24 h using LC-MS technique, and found 37 differential expressed proteins. In them, the expression of 19 proteins were up-regulated such as EEF1A1, and 18 were down-regulated such as CFL1. These results could help us to understand why D-HFt had better effects compared with free doxorubicin, and the molecular mechanism beneath in future studies.

In addition, we constructed nanometer cisplatin core recombinant human H-ferritin (NCC-HFt). We also studied differential expressed proteome after treated HepG2 cells with NCC-HFt or cisplatin 24 h using LC-MS technique, and found 24 differential expressed proteins. In them, the expression of 19 proteins were up-regulated such as AHSG, and 5 were down-regulated such as ALDOA. These results could help us to understand the influence to cells due to slow-release function of NCC-HFt, and the molecular mechanism beneath in future studies.

Key Words: ferritin; targeting drug delivery; proteomics

缩略语

- 2-DE: two-demensional electrophoresis, 双向电泳
- ACN: acetonitrile, 乙腈
- APS: ammonium persulfate, 过硫酸铵
- BSA: bovine serum albumin, 牛血清白蛋白
- CDDP: Cisplatin, 顺铂
- CHAPS: 3-[(3-cholamidopropyl)dimethylammonio] propanesulfonate, 3-[3-(胆酰胺丙基)二甲氨基]丙磺酸内盐
- CHCA: α -cyano-4-hydroxy-cinnamic acid, α -氰-4-羟肉桂酸
- DEPC: iethypyrocarbonate, 焦碳酸二乙酯
- D-HFt: Doxorubicin core recombinant human H-ferritin, 阿霉素核-H 亚基铁蛋白
- DMSO: Dimethyl sulfoxide, 二甲基亚砷
- Dox: Doxorubicin, 阿霉素
- DTT: dithiothreitol, 二硫苏糖醇
- FDR: False discovery rate, 错误发现率
- FITC: fluorescein isothiocyanate, 异硫氰酸荧光素
- GST: Glutathione S-transferase, 谷胱甘肽 S 转移酶
- H&E: Hematoxylin and eosin stain, 苏木精-伊红染色
- HFt: human H-ferritin, 人 H 亚基铁蛋白
- IAA: iodoacetamide, 碘乙酰胺
- IEF: isoelectric focusing, 等电聚焦
- LC-MS: Liquid chromatography - mass spectrometry, 液相色谱-质谱联用
- LC-MS/MS: Liquid chromatography - tandem mass spectrometry, 液相色谱-串联质谱联用
- MALDI-TOF-MS: matrix assisted laser desorption/ionization time of flight mass spectrometry, 基质辅助激光解吸/电离飞行时间质谱

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